Histochem Cell Biol (1997) 107:121-

ORIGINAL PAPER

Michaela Aubele · Horst Zitzelsberger · Sandor Szücs
Martin Werner · Herbert Braselmann · Peter Hutzler
Karsten Rodenacker · Lars Lehmann
Günter Minkus · Heinz Höfler

pd. 1997

Comparative FISH analysis of numerical chromosome 7 abnormalities in 5-μm and 15-μm paraffin-embedded tissue sections from prostatic carcinoma

p. 121-126 (6)

Accepted: 7 November 1996

Abstract Interphase fluorescence in situ hybridization (FISH) was performed on 15-μm-thick paraffin sections from prostatic carcinomas using a chromosome 7-specific α-satellite DNA probe. A confocal laser scanning microscope (CLSM) was used for optical sectioning of the thick sections and reconstruction of 3D images. The number of FISH signals was determined by a gallery of optical sections evaluating only complete nuclei. To investiate the influence of section thickness and truncation and nuclei on scoring results, we compared the FISH data from 15-μm sections with signal counts obtained from 5-μm sections. The latter were evaluated by conventional fluorescence microscopy in the same tumor regions previously defined and marked on the slides. After statistical analysis of spot frequencies in tumor and non-tumorous cells (χ^2 test), we transferred the signal frequencies into a cytogenetic classification (–7, +7, polysomy 7). Based on this classification, most cases showed more than one chromosome 7 aberration type. Trisomy 7 (+7) became apparent in 15-μm-thick sections in all 19 tumors, polysomy 7 (>3 spots) in 18/19 cases, and monosomy 7 (–7) in 13/19 cases. In 5-μm sections, however, trisomy 7 and polysomy 7 were found in only 7/19 and 13/19 cases, respectively, and monosomy 7 in 7/19 cases. When comparing the classification results of tumor cells of the same tumor regions originating either from 5-μm or 15-μm sections, the following discrepancies were noted: in 15-μm sections exclusively, in 12/19 tumors, trisomy 7 was found; in 6/19 cases, polysomy 7; in 8/1' cases, monosomy 7. The high proportion of cases with tumor nuclei expressing only one hybridization signal o chromosome 7 in 15-μm sections could be confirmed a monosomy 7 in five selected cases by double-hybridiza tion using centromere-specific probes for chromosome 7 and 12. These results demonstrate that numerical chromosome 7 aberrations are more frequently observed i thick (15-μm) paraffin-embedded tissue sections by evaluating only complete nuclei. The use of routine section (5-μm) for interphase cytogenetic analyses is compromised by a remarkable underestimation of the real chromosome copy numbers.

M. Aubele (✉) · P. Hutzler · K. Rodenacker · G. Minkus
H. Höfler
GSF - National Research Center for Environment and Health,
Institute of Pathology, Ingolstädter Landstrasse 1,
D-85764 Oberschleissheim, Germany;
fax +49-89-3187 3349

H. Zitzelsberger
Ludwig Maximilians Universität München,
Institute of Radiobiology, Germany

H. Zitzelsberger · H. Braselmann · L. Lehmann
GSF-Institute of Radiology, Germany

S. Szücs · M. Werner · H. Höfler
Technische Universität München, Institute of Pathology, Germany

Introduction

Fluorescence in situ hybridization (FISH) using centromere-specific DNA probes allows the detection of numerical chromosome aberrations in the interphase nucleus and is generally referred to as 'interphase cytogenetics' (Hopman et al. 1991). Several studies have demonstrated the reliability of this technique for an evaluation of numerical chromosome aberrations (Alers et al 1995a; Bandyk et al. 1994; Herrington et al. 1995; Micale et al. 1992). To enable a correlation of FISH analysis and histomorphological patterns, the method was modified for its application on routine paraffin-embedded tissue sections (van Dekken et al. 1992; Dhingra et al 1994; Hopman et al. 1991; Kim et al. 1993). Thus, archival specimens can be examined and retrospective studies can be carried out on tumors from patients with known clinical outcome (Lifson et al. 1995).

Only a few studies of interphase analysis on paraffin-embedded tissue sections from prostatic carcinoma have been published so far, which demonstrated gains and losses of chromosomes 7, 8, 10, 17, X, Y (Alers et al 1995a, b; Baretton et al. 1994; van Dekken et al. 1992 Zitzelsberger et al. 1994). An increased rate of aneuploi-

dy was shown to be associated with a high histological grading (Baretton et al. 1994) and an advanced stage of disease (Henke and Ayhan 1994). Although aneuploidies of several chromosomes were detected in prostate cancer, the presence of numerical chromosome 7 aberrations could be shown to be associated with the development of lymph node metastases and low tumor grade (Zitzelsberger et al. 1994) as well as with a poor prognosis of patients (Alcaraz et al. 1994; Henke et al. 1994).

Despite the great advantage of a correlation between FISH analysis and histological pattern, a remarkable disadvantage when performing this technique on routine 5-µm sections is the considerable amount of sliced nuclei, which may result in an underestimation of the true chromosome copy number (Hopman et al. 1991). A correlation between an average signal count from routine sections and corresponding touch preparations containing predominantly complete nuclei could be demonstrated (Dhingra et al. 1994; Hopman et al. 1992). Furthermore, attempts were made to evaluate the amount of chromosome underestimation due to sectioning by using tissue model systems containing mixtures from cell populations with known chromosome numbers (Dhingra et al. 1994; Hopman et al. 1991). However, there are no clearly defined criteria for quantitative signal analysis that can account for nuclear slicing during sectioning (Dhingra et al. 1994).

To investigate the influence of section thickness and truncation of interphase cell nuclei on scoring results, we performed FISH analyses with an α-satellite DNA probe for chromosome 7 on consecutive 5-µm and 15-µm sections from 19 prostatic carcinomas. Scoring of hybridization signals on 15-µm sections was exclusively performed in uncut, whole nuclei by use of the confocal laser scanning microscope (CLSM) and optical sectioning, whereas 5-µm sections were evaluated by conventional fluorescence microscopy in identical tumor areas.

Materials and methods

Patients and tumor samples

Routinely processed, formalin-fixed and paraffin-embedded tissue specimens from radical prostatectomies of 19 patients with prostatic adenocarcinoma were used in this study. Consecutive sections were made consisting of 5-µm sections for hematoxylin-eosin staining (H&E) or FISH, and 15-µm sections for FISH with subsequent CLSM scoring. Sections were mounted on slides and baked overnight at 37° C for better adherence.

Additionally, for double-hybridization experiments, 30-µm-thick sections were prepared and stained with methylene blue. From these sections, the corresponding tumor areas were selectively cut out with a fine scalpel under microscopic control. For disaggregation of tumor nuclei, the protocol of Alers et al. (1995a) was modified. Briefly, after deparaffination and rehydration, samples were digested in a pronase E solution (0.05% in phosphate-buffered saline, PBS, pH 7.0) for 2 h at 37° C, with vigorous vortexing every 15 min. The cell suspension was then centrifuged, washed twice in cold PBS before filtering through a nylon mesh (pore size 30 µm) to remove any remaining tissue fragments. The cells were resuspended in cold Carnoy's fixative (methanol:acetic acid 3:1) and dropped onto clean slides.

FISH

DNA probes

α-Satellite DNA probes specific for the centromeric region o chromosomes 7 and 12 were generated as described (Zitzelsberge et al. 1994). The probes were biotin- (chromosome 7) and digox genin- (chromosome 12) labeled by nick-translation according t standard procedures. The specificity of the probes was checked o metaphase preparations from peripheral lymphocytes of a health donor. The hybridization mixture consisted of carrier DNA (0. µg/µl herring sperm DAN), mastermix 2.1 (final concentratio 55% formamide, 1×SSC, 10% dextran sulfate) and the labele DNA probes. This mixture was denatured at 72° C for 5 min an chilled on ice until use.

Hybridization

For FISH of 15-µm-thick sections, the protocol according t Zitzelsberger et al. (1994) was slightly modified. The pretreatmen of sections with pronase E solution was performed at 37° C fo 20–40 min. This proteolytic pretreatment step had to be optimize for each paraffin block. Those cases which did not exhibit stron signal throughout the section thickness even after intense proteo lytic digestion were additionally treated by microwave at 750 W for 2 min in a citrate buffer (pH 6.0). After denaturation of th sections in 70% formamide (70% formamide, 2 ×SSC) at 72° C for 20 min, the sections were dehydrated in a series of 70%, 90% and 100% ice-cold ethanol and dried. Denaturation of the slide containing disaggregated nuclei was performed in 70% formamid at 72° C for 3–20 min, the 5-µm paraffin sections were treated a previously described (Zitzelsberger et al. 1994). The hybridizatio mixture (30 µl) was applied to each slide, covered by a glas cover slip, and sealed with rubber cement. After overnight incubation a 37° C, post hybridization washing was performed at 43° C as pre viously described (Zitzelsberger et al. 1994).

Detection and counterstaining

The biotin-labeled centromere 7 probe was detected by streptavidin-fluorescein isothiocyanate (FITC) and biotinylated anti-streptavidin conjugates (Camon, Wiesbaden). Detection of the digoxigenin-labeled probe of centromere 12 was performed by an anti-digoxigenin antibody (Boehringer, Mannheim) and repeated cycles of Cy-3-conjugated rat anti-mouse and mouse anti-rat antibodies (Dianova, Hamburg). After washing, the nuclei were counterstained with propidium iodide (PI, Sigma) in single FISH experiments or with 4'6-diamidino-2-phenylindole (DAPI, Sigma) in double-hybridization experiments, and subsequently mounted with an antifade solution (Johnson and de Nogveira Aranjo 1981).

Evaluation of FISH signals

Image acquisition and evaluation of chromosome 7 signals in 15-µm-thick sections

Comparison of the hybridized sections with the following H&E stained section allowed an unequivocal assignment of cells to tumor or non-tumorous regions. Representative tumor regions with well preserved morphology and unambiguous FISH signals for centromere 7 were scanned using a CLSM 410 (Zeiss, Oberkochen) (D'Alessandro et al. 1996). For the acquisition of the images, a 100×lens was used (Zeiss PNF, NA 1.3, oil immersion). Both, FITC and PI were excited by an Argon laser (488 nm), and the fluorescence was measured simultaneously in two separate channels using the green channel for FITC (515–565 nm) and the red channel for PI (590 nm). From each field of view, up to 32 confocal images were scanned with an axial distance of 0.5 µm between two subsequent images. the scanned field of 62.5×62.5

µm was sampled to 256×256 pixels, giving a resolution of 0.25 µm in the *x* and *y* directions. Between 15 and 20 different fields of view were scanned in each tumor and, in addition, normal epithelium or stromal cells as control. All scanned images were stored on a disk as true-color TIF files.

For evaluation of signals of 15-µm-thick sections, a Unix workstation was used connected via local area network to the PC of the CLSM. Interactive evaluation and storage of signal counts was performed by a graphical user interface, which is partly shown in Fig. 1. It allows the display of the original digitized volume by orthogonal sections (Fig. 1A), selection of a nucleus of interest by cursor and adjustment of a 3D box, and a subsequent display of the selected 3D box as a gallery of optical sections in a separate window (Fig. 1B). In this gallery, the intactness of the nucleus was ascertained, the number of signals associated with the nucleus was determined according to accepted criteria (Hopman et al. 1991), and the signal number for a given nucleus was stored together with the box coordinates. The following criteria were additionally applied for evaluation: (1) only intact nuclei were considered; (2) only signals were counted which could unambiguously be assigned to a nucleus; and (3) only signals which occurred in at least two successive optical sections were counted. From each tumor, the signal frequency of 200 nuclei was determined and stored. In addition, a total of about 500 normal epithelium or stromal cells was evaluated for control.

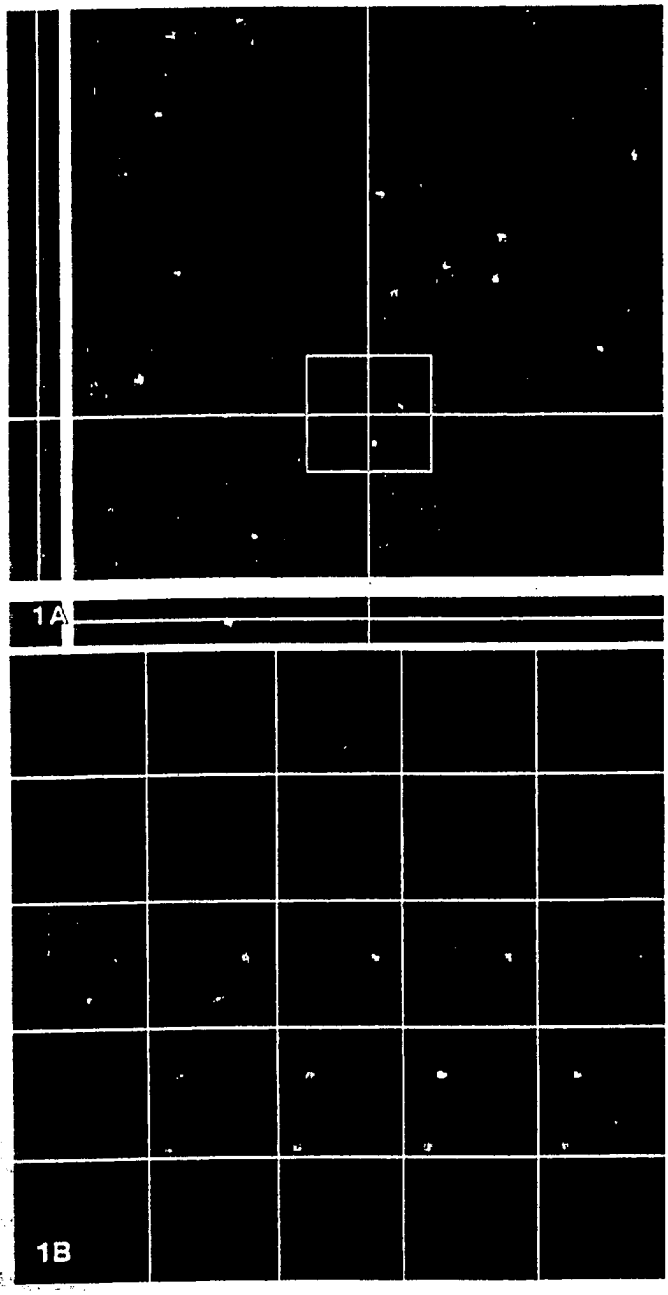

Fig. 1A, B Graphical user interface for evaluation of signals in 15-µm-thick sections. The field of view contains prostatic carcinoma nuclei with fluorescein isothiocyanate (FITC)-detected centromere 7 and propidium iodide counterstaining. **A** Orthogonal representation with the pointer (*crossed lines*) positioned on a nucleus of interest, and a 3D box adjusted to the nucleus. **B** Gallery of optical sections from the selected nucleus, stored linewise from top right to bottom left of all focus planes of the selected box. Three signals belong to the intact nucleus

Evaluation in 5-µm sections and disaggregated material

In 5-µm sections, scoring of chromosome 7 signals was performed by conventional fluorescence microscope in the same tumor regions which had also been analyzed in the neighboring 15-µm section. The chromosome 7 signals of about 200 tumor nuclei were scored as 1, 2, 3, >3 spots per nucleus.

In disaggregated, double-hybridized (chromosomes 7 and 12) tumor cells, between 100 and 200 non-overlapping nuclei with intact morphology (i.e., spherical) were visually counted for each tumor, and the fluorescence signals for both chromosomes were scored (Fig. 2). An excitation of 543 nm was used for centromere 12 (Cy-3, emission 590 nm), excitation 488 nm for centromere 7 (FITC, emission 515–565 nm), and excitation 360 nm for DAPI (emission 400–430 nm).

Statistical analysis

For a control, a total number of about 500 normal ductal epithelium or stromal cells was scored in the same 15-µm and 5-µm sections which had also been analyzed for chromosome 7 signals in tumor nuclei. Distribution of hybridization spots among these normal interphase cell nuclei and the tumor cells was analyzed by the χ^2 test. Statistically significant differences were accepted for *I* values ≤0.01. Based on this statistical evaluation, a cytogenetic

Fig. 2 Representative example of disaggregated tumor nuclei after double-hybridization (centromere 7/FITC, centromere 12/Cy-3, counterstaining with 4′,6-diamidino-2-phenylindole). One nucleus exhibited monosomy for chromosome 7 (*green spot*) and disomy for chromosome 12 (*red spots*)

classification was performed identifying cases with monosomy (one spot), trisomy (three spots), and polysomy 7 (>3 spots).

Results

The frequency distribution of chromosome 7-specific FISH signals in tumor nuclei from 15-µm and 5-µm sections of 19 analyzed cases is shown in fig. 3. Based on the applied statistical evaluation, all 19 cases exhibited

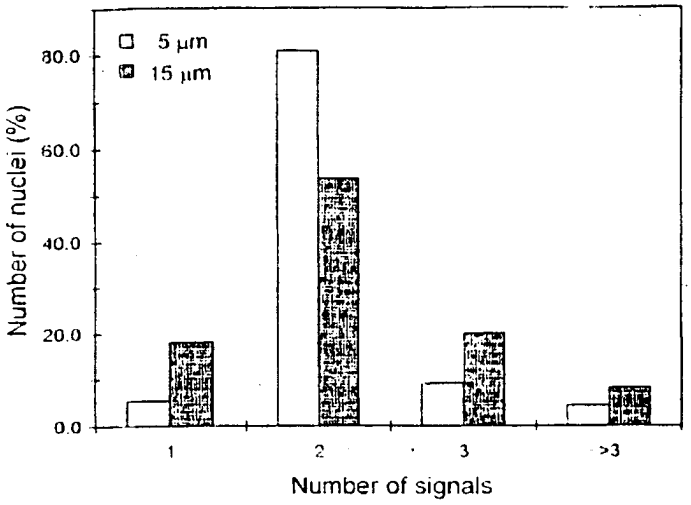

Fig. 3 Frequency distribution of hybridization spot numbers of chromosome 7 from 15-µm and 5-µm sections of 19 prostatic carcinomas

trisomy 7 (three hybridization signals), 18/19 cases polysomy 7 (more than three signals), and 13/19 cases monosomy 7 (one signal) in 15-µm-thick sections (Table 1). 5-µm sections, however, trisomy 7 was found in only 7/19 cases, polysomy 7 in 13/19 cases, and monosomy 7 in 7/19 cases. When comparing cytogenetic findings from 5-µm and 15-µm sections (Table 2), matching results could be obtained for 24 detected aberrations of chromosome 7, but the substantial number of 26 aberrations became exclusively apparent in 15-µm sections. As shown in Table 2, 12 tumors exhibited trisomy 7 exclusively in 15-µm sections, while this aberration type was simultaneously detected in both 5-µm and 15-µm sections in seven cases. Matching results in 5-µm and 15-µm sections were obtained for polysomy 7 in 12 cases, while six tumors revealed this numerical aberration only in 15-µm sections. Monosomy 7 (−7) was visible in both 15-µm and 5-µm sections in five tumors. In two cases monosomy 7 was only detected in 5-µm sections, and in eight cases, it was seen exclusively in 15-µm sections (Table 2). This high proportion of cases exhibiting only one hybridization signal for chromosome 7 in 15-µm sections required the confirmation of monosomy 7 by simultaneous scoring of a second reference chromosome. We therefore performed double-hybridization analyses for chromosomes 7 and 12 in five selected cases (3, 6, 10, 18, 19). To simplify the hybridization and scoring procedure, we disaggregated tumor cells from 30-µm-thick sections from the identical tumor areas from which the 5-µm and 15-µm sections had been prepared. Between 100 and 200 tumor nuclei were scored for both

Table 1 Hybridization signals for chromosome 7 observed in 19 cases of prostatic carcinoma in 15-µm and 5-µm-thick paraffin-embedded tissue sections. Aberrant cases in the tumor cells exhibited significantly higher frequencies for a particular spot number compared to the corresponding non-tumorous cells (+ significantly different from control, − not significantly different, χ^2 test $P \leq 0.01$)

Case number	15-µm sections							5-µm sections						
	Signals per nucleus (%)				Cytogenetic classification			Signals per nucleus (%)				Cytogenetic classification		
	1	2	3	>3	−7	+	Polysomy 7	1	2	3	>3	−7	+7	Polysomy 7
1	3.6	29.9	32.1	34.4	−	+	+	3.5	83.1	4.7	8.7	−	−	+
2	23.3	49.4	19.1	8.2	+	+	+	1.0	81.6	6.0	11.4	−	−	+
3	24.8	45.1	18.9	11.2	+	+	+	6.0	73.3	18.1	2.6	−	+	+
4	24.8	58.9	13.5	2.9	+	+	+	12.1	73.8	10.3	3.7	+	+	+
5	14.6	65.7	19.2	0.5	+	+	−	9.1	84.4	4.5	1.5	+	−	+
6	22.1	44.7	18.4	14.7	+	+	+	0.6	87.8	10.5	1.2	−	+	−
7	21.1	47.1	19.6	12.3	+	+	+	12.0	83.1	4.8	0.0	+	−	−
8	25.5	44.7	26.0	3.8	+	+	+	1.9	82.3	15.8	0.0	−	+	−
9	24.9	49.2	20.5	5.4	+	+	+	7.1	86.3	6.0	0.6	+	−	−
10	26.4	54.1	15.5	4.1	+	+	+	1.7	73.3	15.9	9.1	−	+	+
11	5.4	77.5	14.7	2.5	−	+	+	3.9	91.0	3.9	1.3	−	−	−
12	3.7	53.2	29.4	13.8	−	+	+	5.8	69.0	18.1	7.0	−	+	+
13	7.5	72.1	16.4	4.0	−	+	+	10.5	79.1	7.8	2.6	+	−	+
14	3.9	62.8	25.6	7.8	−	+	+	4.6	89.3	4.1	2.0	−	−	+
15	18.4	44.7	25.2	11.7	+	+	+	3.6	82.1	9.2	5.1	−	−	+
16	11.5	51.6	30.3	6.6	−	+	+	8.2	75.9	9.4	6.5	+	−	+
17	25.4	46.4	21.0	7.2	+	+	+	7.1	80.7	6.1	6.1	+	−	+
18	23.4	64.8	9.8	2.0	+	+	+	3.0	77.2	9.4	10.4	−	−	+
19	29.3	54.0	14.4	3.3	+	+	+	2.3	80.8	16.2	0.8	−	+	−
Total					13/19	19/19	18/19					7/19	7/19	13/19

Table 2 Number of cases exhibiting chromosome 7 aberrations in 5-µm and 15-µm sections (χ^2 test $P \leq 0.01$)

	Number of detected aberrations		
	Matching results in 5-µm and 15-µm sections	Exclusively in 5-µm sections	Exclusively in 15-µm sections
Trisomy 7	7	–	12
Polysomy 7	12	1	6
Monosomy 7	5	2	8

chromosomes. A substantial proportion of interphase cell nuclei exhibited only one hybridization signal for chromosome 7 but two signals for chromosome 12, which confirmed the cytogenetic classification of monosomy 7 in all five cases. The frequencies of nuclei showing one hybridization spot for chromosome 7 in disaggregated tumor material were 18.4% (case 3), 24.1% (case 6), 23.3% (case 10), 21.1% (case 18), and 15.8% (case 19), which were similar to the observed frequencies of nuclei with one hybridization spot in 15-µm sections (Table 1). Figure 2 shows disaggregated and double-hybridized interphase cell nuclei, from which one of them exhibited two red signals for chromosome 12 and one green signal for chromosome 7.

Discussion

Following the modification of FISH for paraffin-embedded sections (van Dekken et al. 1992; Dhingra et al. 1994; Hopman et al. 1991; Kim et al. 1993), archival tissue of different neoplasms could be investigated and a correlation of cytogenetic aberrations with morphological pattern, grade of tumors, and the clinical course of patients could be undertaken (Alers et al. 1995a, b; Arnoldus et al. 1991; Baretton et al. 1994; van Dekken et al. 1992; Zitzelsberger et al. 1994). Quantitative analysis of chromosome copy number in routinely processed sections (5-µm), however, is compromised by the fact that most nuclei are sliced (Dhingra et al. 1994; Hopman et al. 1991). To evaluate the significance of these artefacts of sample preparation for FISH analysis, we performed FISH simultaneously on 5-µm and 15-µm sections and scored hybridization signals on 15-µm sections in complete nuclei using the CLSM and optical sectioning. Applying this technique, a variation of section thickness between 10-µm and 20-µm was observed in 15-µm-cut sections. Depending on the nuclear diameter, beteen 60% and 70% of the nuclei in a field of view were complete. For FISH in 15-µm sections, a refined and optimized procedure had to be established, which provided intense FISH signals throughout the section thickness as well as an intact morphology of tissue sections. From a methodological point of view, FISH on 15-µm sections with subsequent optical sectioning caused several problems. The thicker sections were more often released from the glass slides as a result of digestion and denaturation. As the success rate of FISH is mainly dictated by the accessibility of the chromosomal target DNA, stronger pretreatment is needed to permeabilize the tissue sections to allow penetration of probe and antibodies (Hopman et al. 1994 This strong pretreatment is a compromise between loss c morphology and FISH signal intensity. Furthermore, fc an exact evaluation of signals in thick sections, the CLSI is essential due to out-of-focus fluorescence and uncer tainty of assignment of signals to a specific nucleu (D'Alessàndro et al. 1996; Tekola et al. 1994; Thompso et al. 1994). The successfully established approach c FISH on 15-µm sections and subsequent optical section ing and evaluation of signals by means of a gallery c confocal images is currently limited for routine applica tion by the requirement of technical equipment and th time-consuming scoring of signals; no automatic segmen tation of nuclei in a 3D volume is available, which woul be the prerequisite for automatic signal counting.

A comparative FISH analysis of numerical chromo some 7 aberrations in 5-µm and 15-µm sections was per formed on 19 cases of prostatic cancer. The frequenc distribution of all 19 tumors (Fig. 3) revealed a highe proportion of aberrant cells for chromosome 7 in 15-µn sections. About twice as many tumor nuclei exhibitin three or more signals and surprisingly, a higher frequen cy of cells with only one hybridization signal was detect ed in 15-µm sections. For an identification of aberran cases, a cytogenetic classification was performed fo each single tumor. Based on this classification, variou chromosome 7 aberrations (monosomy, trisomy, poly somy) became apparent in both 5-µm and 15-µm sec tions. Moreover, most cases exhibited more than on chromosome 7 aberration (Table 1). As expected, as consequence of section thickness, trisomy 7 and poly somy 7 were more frequently observed in 15-µm-thick sections. These findings confirm the study of Dhingra e al. (1994) which demonstrated an increasing number o signals per nucleus with an increased section thickness Hopman (1991) showed that, in disaggregated cell mate rial, about 60% of nuclei contained a higher spot numbe than in a corresponding 6-µm section. It was furthe demonstrated by using 6-µm sections from a tissue mod el system, that this underestimation of chromosom numbers parallels an increase of nuclear diameter. As consequence, artefacts of tissue preparation from trun cated interphase cell nuclei will be more pronounced i cells with a higher DNA content because they exhibit larger nuclear diameter. In our study, polysomy 7 and tri somy 7 were exhibited in 6 and 12 cases, respectively exclusively in thick sections. Thus, FISH analysis on 5 µm sections resulted in a remarkable underestimation o chromosome 7 as a result of truncated nuclei. Despit this, in a similar number of cases, matching results be

tween 5-μm and 15-μm sections could be obtained for these abnormalities.

In our study on complete nuclei in 15-μm sections, we also found a greater proportion of tumor cells exhibiting monosomy 7, obviously not present in the corresponding 5-μm sections. In five selected cases, this monosomy 7 was confirmed by double-hybridization with a second centromere-specific DNA probe for chromosome 12. So far, numerical aberrations of chromosome 12 have not been reported for prostatic carcinoma. From our double-hybridization experiments, monosomy 7 could be clearly confirmed due to the high frequency (15–24%) of interphase cell nuclei exhibiting two spots for chromosome 12 but only one spot for chromosome 7. Due to the section thickness one would also expect to detect at least a similar proportion of cases with monosomy 7 in 5-μm sections, but, surprisingly only 7/19 cases showed this aberration. One can speculate that nuclei with monosomy 7 were truncated in 5-μm sections, resulting in an artificial nullisomy. These cells, however, wer not counted since they exhibited no hybridization signal.

In conclusion, our results show that numerical chromosome 7 aberrations could be more precisely detected when performing FISH on thick (15-μm) paraffin-embedded tissue sections, and scoring of signals only in complete nuclei using the CLSM and optical sectioning. The approach described here is currently limited for routine application by the requirement of technical equipment and the time-consuming scoring procedure. However, our results demonstrated that FISH analysis on 5-μm sections is compromised by a remarkable underestimation of the real chromosome copy number and, thus, limits interpretation.

Acknowledgements The authors wish to thank Sabine Holthaus for technical assistance, Ernst Mannweiler for data handling, and Andreas Mair for help in preparation of the illustrations. This work was financially supported by grants from the Deutsche Forschungsgesellschaft (DFG), contract SZ 53/2-1.

References

Alcaraz A, Takahashi A, Brown JA, Herath JF, Bergstralh EJ, Larson-Keller JJ, Lieber MM, Jenkins RB (1994) Aneuploidy and aneusomy of chromosome 7 detected by fluorescence in situ hybridization are markers of poor prognosis in prostate cancer. Cancer Res 54:3998–4002

Alers JC, Krijtenburg P-J, Vissers KJ, Krishnadath SK, Bosman FT, Dekken H van (1995a) Interphase in situ hybridization to disaggregated and intact tissue specimens of prostatic adenocarcinomas. Histchem Cell Biol 104:479–486

Alers JC, Krijtenburg PJ, Vissers CJ, Bosman FT, Kwast TH van der, Dekken H van (1995b) Cytogenetic heterogeneity and histologic tumor growth patterns in prostatic cancer. Cytometry 21:84–94

Arnoldus EPJ, Dreef EJ, Noordermeer IA, Verheggen MM, Thierry RF, Peters ACB, Cornelisse CJ, Ploeg M van der, Raap AK (1991) Feasibility of in situ hybridisation with chromosome specific DNA probes on paraffin wax embedded tissue. J Clin Pathol 44:900–904

Bandyk MG, Zhao L, Troncoso P, Pisters LL, Palmer JL, Eschenbach AC von, Chung LWK, Liang JC (1994) Trisomy 7: a potential cytogenetic marker of human prostate cancer progression. Genes Chromosom Cancer 9:19–27

Baretton GB, Valina C, Vogt T, Schneiderbanger K, Diebold J, Löhrs U (1994) Interphase cytogenetic analysis of prostatic carcinomas by use of non-isotopic in situ hybridization. Cancer Res 54:4472–4480

D'Alessandro I, Zitzelsberger H, Hutzler P, Lehmann L, Braselmann H, Chimenti S, Höfler H (1996) Numerical aberration of chromosome 7 detected in paraffin-embedded tissue sections (15-μm) of primary cutaneous melanomas by fluorescence in situ hybridization and confocal laser scanning microscopy. J Cutan Pathol (in press)

Dekken H van, Kerstens HMJ, Tersteeg TA, Verhofstad AA, Vooijs GP (1992) Histological preservation after in situ hybridization to archival solid tumor sections allows discrimination of cells bearing numerical chromosome changes. J Pathol 168:317–324

Dhingra K, Sneige N, Pandita TK, Johnston DA, Lee JS, Eman K, Hortobagyi GN, Hittelman WN (1994) Quantitative analysis of chromosome in situ hybridization signal in paraffin-embedded tissue sections. Cytometry 16:100–112

Henke R-P, Ayhan N (1994) Enhancement of hybridization efficiency in interphase cytogenetics on paraffin-embedded tissue sections by microwave treatment. Anal Cell Pathol 6:319–325

Henke R-P, Krüger E, Ayhan N, Hübner D, Hammerer P (1994) Frequency and distribution of numerical chromosomal aberrations in prostatic cancer. Hum Pathol 25:476–484

Herrington CS, Cooper K, McGee JOD (1995) Interphase cytogenetics: analysis of numerical chromosome aberrations in isolated cells. J Pathol 175:283–295

Hopman AHN, Hooren E van, Kaa CA van de, Vooijs PGP, Ramaekers FCS (1991) Detection of numerical chromosome aberrations using in situ hybridization in paraffin sections of routinely processed bladder cancers. Mod Pathol 4:503–513

Hopman AHN, Poddighe P, Moesker O, Ramaekers FCS (1992) Interphase cytogenetics: an approach to the detection of genetic aberrations in tumours. In: Herrington CS, McGee JOL (eds) Diagnostic molecular pathology. A practical approach vol 1. Oxford Press, Oxford, pp 141–167

Hopman AHN, Voorter CEM, Ramaekers FCS (1994) Detection of genomic changes in cancer by in situ hybridization. Mol Biol Rep 19:31–44

Johnson GD, Nogueira Araujo GM de (1981) A simple method of reducing the fading of immunofluorescence during microscopy. Immunol Methods 43:349–350

Kim SY, Lee JS, Ro JY, Gay ML, Hong WK, Hittelman WN (1993) Interphase cytogenetics in paraffin sections of lung tumors by non-isotopic in situ hybridization. Am J Pathol 142 307–317

Lifson B, Pizzolo JG, Elhosseiny AA, Choudhury MS, Melamed MR (1995) Comparison of fluorescence in situ hybridization and flow cytometric DNA ploidy analysis in paraffin-embedded prostatic adenocarcinoma specimens. Anal Quant Cytol Histol 17:93–99

Micale MA, Mohamed A, Sakr W, Powell IJ, Wolman SR (1992) V. Cytogenetics of primary adenocarcinoma. Cancer Genet Cytogenet 61:165–173

Tekola P, Zhu Q, Baak JPA (1994) Confocal laser microscopy and image processing for three-dimensional microscopy: technical principles and an application to breast cancer. Hum Pathol 25:12–21

Thompson CT, LeBoit PE, Nederlof PM, Gray JW (1994) Thick section fluorescence in situ hybridization on formalin-fixed paraffin-embedded archival tissue provides a histogenetic profile. Am J Pathol 144:237–243

Zitzelsberger H, Szücs S, Weier H-U, Lehmann L, Braselmann H, Enders S, Schilling A, Breul J, Höfler H, Bauchinger M (1994) Numerical abnormalities of chromosome 7 in human prostate cancer detected by fluorescence in situ hybridization (FISH) on paraffin-embedded tissue sections with centromere specific DNA probes. J Pathol 172:325–335